

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 3

IN THE CLAIMS:

The claims as currently presented and under consideration, are presented below for the Examiner's convenience and to comply with 37 CFR §1.121. This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended): A process for liquefying starch comprising
contacting an aqueous slurry or solution of the starch having a pH between 3.0 and 4.5 with a single addition of a thermostable, acid-stable alpha-amylase obtained by culturing *Bacillus acidocaldarius* ~~with an aqueous slurry or solution of the starch having a pH as low as 3.0~~, the contacting occurring at an elevated a temperature from about 90-155° C without adjusting the pH of the slurry or solution of the starch, and
producing a liquefact having a pH of about 4.0 to 4.5 and DE of about 10-12 within 60-75 minutes after adding the amylase.

2. (Original): The process of claim 1 wherein the contacting occurs without adding a calcium salt to the slurry or solution.

Claim 3 – 5 (Cancelled)

6. (Previously Amended) The process of claim 1, wherein the contacting act further comprises maintaining the contact at the elevated temperature for about 5-8 minutes.

7. (Previously Amended) The process of claim 1 wherein the elevated temperature is about 105-110° C.

8. (Original): The process of claim 7 wherein the contacting act occurs as a single liquefaction step.

9. (Original): The process of claim 8 further comprising providing at least about 150 ASAA units/g of the thermostable, acid-stable alpha-amylase.

GC695 ROA 2005-03-22

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 4

10. (Previously Amended) The process of claim 1 further comprising, prior to the act of contacting, cooking the aqueous slurry or solution of the starch between about 140-155° C for about 5-8 seconds and then reducing the temperature of the cooked slurry or solution to about 90-98° C.

11. (Original): The process of claim 10 wherein the contacting occurs between the thermostable, acid-stable alpha-amylase and the cooked slurry or solution at about 90-98° C for about 60-90 minutes.

12. (Original): The process of claim 11 wherein the contacting act occurs as a single liquefaction step.

13. (Original): The process of claim 12 further comprising providing 1.0 to 10 ASAA units/g of the thermostable, acid-stable alpha-amylase.

14. (Previously Amended) The process of claim 1, wherein the contacting further comprises first and second contacting acts, the first contacting act occurring at about 105-110° C for 5-8 minutes, and the second contacting act occurring at about 95-98° C.

15. (Original): The process of claim 14 further comprising utilizing about 10-35 ASAA units/g of the alpha-amylase in the first contacting act and utilizing about 1-10 ASAA units of the alpha-amylase in the second contacting act.

16. (Original): The process of claim 14 wherein the first and second contacting acts occur as two liquefaction steps.

17. (Previously Amended) A process for liquefying a starch slurry comprising the following acts:

- (a) providing a thermostable, acid-stable α -amylase prepared from a *Bacillus acidocaldarius* species;
- (b) adding the alpha-amylase to a starch slurry having a pH between about 3.0 – 5.0; and

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 5

(c) heating a mixture obtained from step (b) to at least 90° C until a starch liquefact having a pH of about 4.0-4.5 and a DE of about 10-12 DE is obtained within 60 to 75 minutes after adding the amylase,
wherein acts (b) and (c) occur without adjusting the pH of the starch slurry.

18. (Original): The process of claim 17 wherein acts (b) and (c) occur without adding a calcium salt to the starch slurry.

Claims 19 - 20 (Cancelled):

21. (Previously Amended): The process of claim 17 wherein act (c) occurs at about 90-155° C.

22. (Currently Amended): The process of claim 17 wherein act (c) further comprises heating the mixture for about 5-8 minutes to a temperature of between 107-110°C after obtaining said DE of about 10-12.

23. (Original): The process of claim 22 wherein the mixture is heated to about 105-110° C in act (c).

24. (Original): The process of claim 23 wherein act (c) occurs as a single liquefaction step.

25. (Original): The process of claim 24 wherein act (b) further comprises adding at least about 140 ASAA units/g of the thermostable, acid-stable alpha-amylase to the starch slurry.

26. (Original): The process of claim 17 further comprising prior to act (b), the act of cooking the starch slurry between about 140-155° C for about 5-8 seconds and then reducing the temperature of the cooked slurry or solution to about 90-98° C prior to adding the alpha-amylase.

27. (Original): The process of claim 26 wherein act (c) further comprises holding the mixture at about 90-98° C for about 60-90 minutes.

GC695 ROA 2005-03-22

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 6

28. (Original): The process of claim 27 wherein act (c) occurs as a single liquefaction step.

29. (Original): The process of claim 28 wherein act (b) further comprises adding 1.0 to 5.0 ASAA units/g of the thermostable, acid-stable alpha-amylase.

30. (Original): The process of claim 17 wherein act (c) comprises two heating acts, a first heating act occurring at about 105-110° C for 5-8 minutes, and a second heating act occurring at about 95-98° C.

31. (Original): The process of claim 30 wherein act (b) further comprises adding about 10-35 ASAA units/g of the alpha-amylase, and act (c) further comprises adding 1-10 ASAA units of the alpha-amylase in the second heating act.

32. (Original): The process of claim 30 wherein the first and second heating acts occur as two liquefaction steps.

33. (Currently Amended): A single liquefaction step process for starch comprising the following acts:

- (a) providing a starch slurry having a between as low as 3.0 and 4.5 and a thermostable, acid-stable alpha-amylase obtained from *Bacillus acidocaldarius*;
- (b) mixing the alpha-amylase and the starch slurry; and
- (c) heating the resulting mixture to at least 90° C until a liquefact having a pH of about 4.0 to 4.5 and a DE of about 10-12 DE is obtained within 60 to 75 minutes after mixing the amylase,

wherein acts (b) and (c) occur without adjusting the pH of the starch slurry.

34. (Original): The process of claim 33 wherein acts (b) and (c) occur without adding a calcium salt to the starch slurry.

Claims 35 – 36 (Cancelled):

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 7

37. (Original): The process of claim 33 wherein act (c) occurs at about 90-155° C.

38. (Currently Amended) The process of claim 33 wherein act (c) further comprises heating the mixture for about 5-8 minutes to a temperature of between 107-110°C after obtaining said DE of about 10-12.

39. (Original): The process of claim 38 wherein the mixture is heated to about 105-110° C in act (c).

40. (Original): The process of claim 39 wherein act (b) further comprises adding at least about 140 ASAA units/g of the thermostable, acid-stable alpha-amylase to the starch slurry.

41. (Original): The process of claim 33 further comprising prior to act (b), the act of cooking the starch slurry between about 140-155° C for about 5-8 seconds and then reducing the temperature of the cooked slurry or solution to about 90-98° C prior to adding the alpha-amylase.

42. (Original): The process of claim 41 wherein act (c) further comprises holding the mixture at about 90-98° C for about 60-90 minutes.

43. (Currently Amended): A starch liquefaction method comprising the acts of:

- (a) providing a starch slurry having a pH between as low as 3.0 and 4.5 and an thermostable, acid-stable alpha-amylase capable of hydrolyzing starch at a pH as low as 3.0, the alpha-amylase cultured from *Bacillus acidocaldarius*;
- (b) mixing the starch slurry and about 150 ASAA units/g of the alpha-amylase without adjusting the pH of the starch slurry; and
- (c) maintaining the mixture of step (b) at 95°C for about 90 – 120 minutes;
and
- (ed) jet cooking the mixture resulting from step (c) for about 5-8 minutes at about 105-110°C to obtain a liquefact having a DE of approximately 10-12 within 60-75 minutes after mixing the amylase.

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 8

44. (Currently Amended): A starch liquefaction method comprising the acts of:

- (a) providing a starch slurry having a pH between as low as 3.0 and 4.5 and ~~an thermostable, acid-stable alpha-amylase capable of hydrolyzing starch at a pH as low as 3.0, the alpha-amylase cultured from *Bacillus acidocaldarius*;~~
- (b) jet cooking the starch slurry between about 140-155° C for about 5-8 seconds without adjusting the pH of the starch slurry;
- (c) lowering the temperature of the cooked slurry from step (b) to about 95-98 °C and adding about 1.0 to 5.0 ASAA units/g ds of the alpha-amylase; and
- (d) allowing the mixture of step c) to undergo hydrolysis for about 60-90 minutes to produce a liquefact having a DE of about 10-12 within 60 to 75 minutes after adding the amylase.

45. (Currently Amended): A starch liquefaction method comprising the acts of:

- (a) providing a starch slurry having a pH between as low as 3.0 and 4.5 and an thermostable, acid-stable alpha-amylase capable of hydrolyzing starch at a pH as low as 3.0, the alpha-amylase cultured from *Bacillus acidocaldarius*;
- (b) mixing the starch slurry with about 10-30 ASAA units/g ds of the alpha-amylase without adjusting the pH of the starch slurry;
- (c) jet cooking the mixture resulting from step (b) for about 5 minutes;
- (d) adding about 1-5 ASAA units/g ds of the alpha-amylase to the jet cooked mixture from step (c); and
- (e) continuing liquefaction at about 95-98 °C for as little as 30-90 minutes to obtain a liquefact having a DE of approximately 10-12 within 60-75 minutes after adding the amylase;
- (f) jet cooking the mixture resulting from step (e) for about 1-2 minutes.

46. (Previously Amended) A starch liquefact made by the process of claim 1, the liquefact characterized as having a pH of about 4.0 to 4.5, free of maltulose and

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 9

suitable for saccharification without inactivation of the thermostable, acid-stable alpha-amylase and without adjustment of the about 4.0 – 4.5 pH of the liquefact.

47. (Previously Amended) A starch liquefact made by the process of claim 17, the liquefact characterized as having a pH of about 4.0 to 4.5, free of maltulose and suitable for saccharification without inactivation of the thermostable, acid-stable alpha-amylase and without adjustment of the about 4.0 – 4.5 pH of the liquefact.

48. (Previously Amended) A starch liquefact made by the process of claim 33, the liquefact characterized as having a pH of about 4.0 to 4.5, free of maltulose and suitable for saccharification without inactivation of the thermostable, acid-stable alpha-amylase and without adjustment of the about 4.0 – 4.5 pH of the liquefact.

49. (Previously Amended) A starch liquefact made by the process of claim 43, the liquefact characterized as having a pH of about 4.0 to 4.5, free of maltulose and suitable for saccharification without inactivation of the thermostable, acid-stable alpha-amylase and without adjustment of the about 4.0 – 4.5 pH of the liquefact .

50. (Previously Amended) A starch liquefact made by the process of claim 44, the liquefact characterized as having a pH of about 4.0 to 4.5, free of maltulose and suitable for saccharification following thermal inactivation of the thermostable, acid-stable alpha-amylase in the liquefact and without adjustment of the about 4.0 – 4.5 pH of the thermally inactivated liquefact .

51. (Previously Amended) A starch liquefact made by the process of claim 45, the liquefact characterized as having a pH of about 4.0 to 4.5, free of maltulose and suitable for saccharification following thermal inactivation of the thermostable, acid-stable alpha-amylase in the liquefact and without an adjustment of the about 4.0 – 4.5 pH of the thermally inactivated liquefact.

Claim 52. Cancelled.

53. (New) A process for producing glucose from starch comprising the acts of:

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 10

- a) providing a mixture of a starch slurry having a pH between 3.0 and 4.5;
- b) adding a thermostable, acid-stable alpha-amylase capable of hydrolyzing starch at a pH between 3.0 and 4.5, the alpha-amylase cultured from *Bacillus acidocaldarius*;
- c) liquefying the starch slurry by heating the mixture until a DE of about 10-12 is reached within 60 to 90 minutes after the addition of the alpha amylase without the production of maltulose; and
- d) adding a saccharification enzyme to the liquefied starch slurry from step c and maintaining a resulting saccharification mixture at about 60° C for between about 10-48 hours or until about a 95% glucose yield is achieved.

54. (New) The process of claim 53 wherein act a) is carried out without adjusting the pH of the starch slurry.

55. (New) The process of claim 53 wherein act a) is carried out without adding a calcium salt.

56. (New) The process of claim 53 wherein act a) is carried out without adjusting the pH of the starch slurry and without adding a calcium salt.

57. (New) The process of claim 54 wherein act b) further comprises heating the mixture at about 105-110° C for 5-8 minutes.

58. (New) The process of claim 57 wherein act c) is carried out without inactivating the alpha-amylase and without adjusting the pH of the liquefied starch slurry.

59. (New) The process of claim 58 wherein act c) further comprises adding glucoamylase to the liquefied starch slurry.

60. (New) The process of claim 58 wherein act c) further comprises adding a mixture of glucoamylase and pullulanase to the liquefied starch slurry.

Appl. No. 10/026,753
Amdt. dated March 22, 2005
Reply to Office action of September 22, 2004

Page 11

61. (new) The process of claim 53 wherein the pH is between about 4.0 and 4.5.

GC695 ROA 2005-03-22